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Fatty acid profiles, growth, and immune responses of neonatal lambs fed milk replacer and supplemented with fish oil or safflower oil☆

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ABSTRACT

Diets supplemented with long chain, $n-3$ polyunsaturated fatty acids (PUFA) have improved the health and performance of neonatal and growing animals. This study was conducted with lambs that were orphaned at approximately 1 day of age to determine whether supplementing milk replacer fed lambs with oils rich in long chain $n-3$ or $n-6$ PUFA would alter plasma lipid profiles and affect growth characteristics and immune functions. From days 1 to 28 of age, lambs had ad libitum access to commercial milk replacer. From days 7 to 28 of age, lambs received twice daily either 1 g of soybean oil, 1 g of fish oil, or 1 g of safflower oil per os in a gelatin capsule ($n=60$ pens; 20 pens/treatment; one ewe and one ram with similar initial body weights/pen). On days 7, 14, 21, and 28 of age, lambs were weighed, and jugular blood was collected from ram lambs. Lymphocyte proliferation in vitro, differential white blood cell (WBC) counts, and weight gains were quantified. Plasma from days 7 and 28 was used for fatty acid analyses. Fish oil increased ($P<0.001$) plasma total $n-3$ fatty acid concentration and total $n-3$:total $n-6$ fatty acid ratio. Pen body weight (i.e., total lamb weight per pen) increased ($P<0.001$) with day (day 7, 11.9 kg; day 14, 15.1 kg; day 21, 18.2 kg; and day 28, 21.2 kg), but oil treatment did not affect pen body weight. Neither oil treatment, day, nor oil treatment × day interaction were significant for pen body weight gains (3.5 kg), pen average daily gains (0.5 kg), pen milk intakes (19.0 kg), or pen gain:feed ratio (0.18) measured during three intervals: days 7–14; days 14–21; and days 21–28. Day, but not oil treatment, affected ($P<0.001$) unstimulated, concanavalin A stimulated, and lipopolysaccharides stimulated lymphocyte proliferation: days 14, 21, and 28 proliferation > day 7 proliferation. For neutrophils per 100 WBC, the treatment × day interaction was significant ($P<0.05$). Oil treatment and day affected ($P<0.01$ and <0.05, respectively) lymphocyte numbers per 100 WBC. For monocytes, eosinophils, and basophils, neither oil treatment, day, nor the oil treatment × day interaction were significant. Fish oil altered plasma fatty acid profiles, but it did not seem to improve measures of the performance or immune function of healthy, milk replacer fed lambs.

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1. Introduction

Diets that were supplemented with long chain, $n-3$ (e.g., marine fish oil) polyunsaturated fatty acids (PUFA) have improved the health and performance of neonatal and growing animals, although responses to $n-3$ PUFA supplementation have not always been consistent. The latency period between birth and successful suckling was reduced for neonatal lambs that were born to ewes that had been supplemental with fish oil during pregnancy (Capper et al., 2006). In one study, birth weights of lambs born to ewes that were supplemented with fish oil during pregnancy were greater than those for lambs born to control ewes, whereas, in an another study, birth weights and growth rates of lambs from fish oil treated ewes were less than those of controls (Capper et al., 2005, 2007). Pigs born to sows that were supplemented with fish oil during pregnancy had greater birth weights in one study, but not in another, grasped the teat sooner after birth, were less likely to die before weaning, and grew faster during the first 35 days of life than did pigs from control sows (Rooke et al., 2001a,b; Mitre et al., 2005).

Fish oil supplementation increased antibody titers of chicks that were challenged with sheep red blood cells (Fritsche et al., 1991), and the rate of immune system maturation seemed to be enhanced in human infants that were supplemented with fish oil (Damsgaard et al., 2007). In gnotobiotic pigs, fish oil supplementation enhanced the effects of a probiotic, *Lactobacillus paracasei* (Bomba et al., 2003). In addition, fish oil supplementation seemed to reduce the inflammatory response in pigs and other animals, and this may benefit growth performance (Carroll et al., 2003; Gaines et al., 2003; Liu et al., 2003; Mills et al., 2005; Gabler and Spurlock, 2008).

In the western United States, where sheep are raised on rugged and remote rangelands, litters are commonly reduced to two lambs. This practice is called "orphaning." At the U.S. Sheep Experiment Station, approximately 10% of the lambs born annually are orphaned. Orphaned lambs are typically fed milk replacer until they can be transitioned to solid feed. Morbidity and mortality rates of orphaned lambs is thought to be greater than that of lambs raised with their dams, but the true health status and survival of orphaned lambs in the western United States is not known. Nevertheless, interventions that would improve the health and survival rates of orphaned lambs would benefit the lambs and sheep producers.

Table 1
Manufacturer's guaranteed analysis of milk replacer[a]

Manufacturer's guaranteed analysis, DM basis	
Crude protein (%)	24.00
Crude fat (%)	35.00
Crude fiber (%)	0.15
Ca (%)	0.40–0.90
P (%)	0.40
Cu (ppm)	5.00–10.00
Se (ppm)	0.30
Vitamin A (IU/kg)	44000.00
Vitamin D_3 (IU/kg)	11000.00
Vitamin E (IU/kg)	220.00

[a] Ultra Fresh Lamb Milk Replacer, Land O'Lakes, Fort Dodge, IA, USA.

Even though scientific literature contains numerous examples of potential benefits of fish oil treatment on neonatal health and performance, the lack of consistency in results makes it difficult to determine whether fish oil supplementation is actually beneficial. Because of the reported effects of fish oil on immune functions and performance of young animals, we conducted this study to determine whether supplementing orphaned, milk replacer fed lambs with oils rich in long chain $n-3$ (i.e., fish oil) and $n-6$ (i.e., safflower oil) PUFA would alter plasma lipid profiles and affect growth characteristics and immune functions.

2. Materials and methods

2.1. Lambs

The USDA, Agricultural Research Service, U.S. Sheep Experiment Station, Institutional Animal Care and Use Committee approved all animal protocols used for this study. Lambs that were orphaned at approximately 1 day of age were assigned to this experiment. An orphaned lamb is one that is taken from its dam soon after birth to be reared artificially. When litter size is greater than two lambs, orphaning is a common management practice at the U.S. Sheep Experiment Station. Orphaned lambs are typically sold as surplus to commercial sheep producers with the resources to artificially rear the lambs, rather than raised at the U.S. Sheep Experiment Station. The lambs that were assigned to this experiment were orphaned for one or more of the following reasons: a ewe died soon after parturition and her lamb or lambs could not be fostered to another ewe or ewes; a ewe produced multiple lambs but did not seem able to produce enough milk for all of the lambs; a lamb was considerably smaller than a twin or the other lambs in a litter. All of the lambs assigned to this experiment were alert and apparently healthy, and there were no signs of malnutrition, pneumonia, diarrhea, or increased body temperature when the lambs were orphaned.

2.2. Artificial rearing and diet

Artificial rearing programs typically include a period of milk replacer feeding, a period of transition to solid feed, and then termination of milk replacer feeding (Umberger, 1997). This experiment was conducted during the period of milk replacer feeding. From birth to approximately 24 h of age, lambs that were used for this experiment were allowed to suckle their dams and receive colostrum. During that period, each lamb was examined, weighed, and ear tagged, and an elastrator band was positioned slightly distal to the caudal tail fold to initiate tail docking.

At approximately 24 h of age, lambs were removed from their dams and moved to pens inside one room in a heated, ventilated barn. The pens were constructed of solid, opaque, chemical resistant, stress relieved polypropylene sheets (HPG International, Mountain Top, PA, USA), were 117 cm long × 117 cm wide × 61 cm high, and were positioned over an expanded metal floor. Each pen adjoined at least one other pen, and lambs had nose-to-nose contact with lambs in the adjoining pen or pens. One ram lamb and one ewe lamb were assigned to each of 60 pens. The lambs were matched according to time of birth and weight at birth to minimize the initial differences, and possibly competition, between the two lambs.

Our artificial rearing methods are based on information in an agricultural extension service bulletin (Umberger, 1997). From days 1 to 28 of age, lambs had ad libitum access to milk replacer (Ultra Fresh Lamb Milk Replacer, Land O'Lakes, Fort Dodge, IA, USA), via three nippled buckets (Premier 1, Washington, IA, USA). Table 1 contains the manufacturer's guaranteed analysis for the milk replacer. Table 2 contains the average concentrations of various fatty acids in milk replacer that we sampled from four bags of the product; fatty acids were quantified with procedures that have been described previously (Loor et al., 2002). Each sample was collected just after a bag was opened, and samples were collected at approximately weekly intervals during the experiment. The milk replacer was prepared and fed according to the manufacturer's instructions. Buckets and nipples were cleaned and sanitized daily. Consumption of milk replacer per pen was measured once daily. Water was frozen in 2 L bottles, and a bottle of the frozen water was placed periodically into each nippled bucket to keep the milk replacer cool. Cool milk replacer does not

Table 2
Fatty acid concentrations (μg/mg of sample) in samples from four of the 11.3-kg bags of milk replacer that were used in this experiment

Fatty acid	Concentration
4:0	0.17
6:0	0.07
8:0	0.02
10:0	0.18
$C_{12:0}$	0.19
$C_{14:0}$	1.90
$C_{14:1}$	0.05
$C_{15:0}$	0.12
$C_{16:0}$	36.47
trans $C_{16:1}$	0.04
$C_{16:1}$	3.21
$C_{17:0}$	0.49
$C_{18:0}$	18.98
trans-6 $C_{18:1}$ or *trans*-7 $C_{18:1}$[a]	0.10
trans-9 $C_{18:1}$	0.19
trans-10 $C_{18:1}$	0.21
trans-11 $C_{18:1}$	0.17
trans-16 $C_{18:1}$	0.04
trans-12 $C_{18:1}$ or *cis*-7 $C_{18:1}$[a]	0.04
trans-13 $C_{18:1}$ or *cis*-6 $C_{18:1}$[a]	0.09
cis-9 $C_{18:1}$	57.79
cis-11 $C_{18:1}$	3.90
cis-12 $C_{18:1}$	0.24
cis-13 $C_{18:1}$	0.23
cis-15 $C_{18:1}$	0.12
$C_{18:2(n-6)}$	25.14
trans-9, *trans*-12 $C_{18:2}$	0.03
trans-12, *cis*-9 $C_{18:2}$	0.11
trans-9, *cis*-12 $C_{18:2}$	0.04
trans-11, *cis*-15 $C_{18:2}$	0.01
cis-9, *cis*-11 $C_{18:2}$	0.03
cis-9, *trans*-11 CLA[b]	0.21
trans-11, *trans*-13 CLA[b]	0.03
Other *trans*, *trans* CLA[b]	0.04
$C_{18:3(n-3)}$	1.37
$C_{20:0}$	0.32
$C_{20:3(n-3)}$	0.15
$C_{20:4(n-6)}$	0.35
$C_{20:5(n-)}$	0.02
$C_{22:1}$	0.15
$C_{22:4(n-3)}$	0.16
$C_{22:5(n-3)}$	0.10
$C_{22:6(n-3)}$	0.04
Total	153.30

[a] Fatty acids coeluted, and the peak could have been either one of the two.

[b] CLA = conjugated linoleic acid. All of these are $C_{18:2}$ lipids.

reduce lamb growth performance, keeps milk replacer fresher after it has been dispensed, and reduces labor (Brisson and Bouchard, 1970).

2.3. Soybean oil, fish oil, or safflower oil supplementation

From days 7 to 28 of age, lambs in each pen received one of three treatments: 1 g twice daily (morning and evening) of either soybean oil (control and to keep diets isocaloric; Fisher Scientific, Pittsburgh, PA, USA), fish oil (source of $n-3$ fatty acids; Omega Protein, Houston, TX, USA), or safflower oil (source of $n-6$ fatty acids; Ventura Foods, Opelousas, LA, USA) per os in a gelatin capsule (20 pens/treatment; both lambs in a pen received the same treatment). Pill forceps were used to deposit gelatin capsules over the back of the tongue of each lamb so that the lambs swallowed, but did not chew, the capsules.

2.4. Body weights, blood samples, and fatty acid analyses

On days 7, 14, 21, and 28 of age, lambs were weighed, and 10 mL jugular blood samples were collected from each ram lamb. Because of the number of blood samples that we were able to process in a day, blood samples were not collected from ewe lambs. On day 28 of age, the experiment was terminated, and the process of transitioning lambs from milk replacer to solid feed was initiated. Because of signs of poor health, 7 of the initial 120 lambs (5.8%) were removed from the experiment. Four of the lambs were from the soybean oil and three were from the safflower oil group, and not more than one lamb was removed from any of the pens. Based on unpublished U.S. Sheep Experiment Station data for 70,695 lambs born during a 16-year period ending in 2007, the mortality rate for lambs, of ages comparable to those used for this experiment, that were reared with their dams was approximately 5.5%, which is comparable to the removal rate in this experiment.

Jugular blood samples were collected into Becton Dickinson CPT cell preparation tubes (Becton Dickinson and Company, Franklin Lakes, NJ, USA) and centrifuged within approximately 1 h after collection. Lymphocytes were harvested from all blood samples for lymphocyte proliferation assays. Plasma from the samples collected on days 7 and 28 was stored at −20 °C and used later for fatty acid analyses (Loor et al., 2002).

2.5. Lymphocyte culture and white blood cell counts

Lymphocyte isolation and culture procedures were the same as those we have used previously (Lewis and Wulster-Radcliffe, 2006). A non-radioactive method was used according to the manufacturer's instructions to quantify lymphocyte proliferation (CellTiter 96 AQ_{ueous} Non-Radioactive Cell Proliferation Assay; Promega, Madison, WI, USA). Lymphocytes were cultured without and with mitogens to determine unstimulated and stimulated proliferation. Concanavalin A (Con A; stimulates T cells; 1.0 μg/well; Sigma–Aldrich, St. Louis, MO, USA) and lipopolysaccharides (LPS; stimulates B cells; 0.5 μg/well; Sigma–Aldrich) were the mitogens (Lewis and Wulster-Radcliffe, 2006). The data were expressed as optical density units (odu).

Before centrifugation, a few drops of each blood sample were used to prepare slides for differential white blood cell (WBC) counts. Wright stain and a light microscope with an oil immersion objective were used for WBC counts. The morphological characteristics of the cells were used to estimate the numbers of lymphocytes, neutrophils, monocytes, eosinophils, and basophils/100 WBC (Wulster-Radcliffe et al., 2005).

2.6. Statistical methods

Mixed models procedures were used to analyze the data (Proc Mixed; SAS, Cary, NC, USA). Model syntax included the repeated option to account for repeated measures. Except for the analysis to determine the effect of gender on weight gain, the models included terms for the fixed effects of treatment, day, and the treatment × day interaction; subject, or experimental unit, was pen nested within treatment. The model to determine the effect of gender on weight gain included terms for the fixed effects of treatment, day, gender, treatment × day, treatment × gender, gender × day, and treatment × gender × day; subject was pen nested within treatment × gender. Unstructured, compound symmetry, first order autoregressive, and heterogeneous first order autoregressive covariance structures were used to model the errors within experimental units across days. Bayesian information criteria were used to determine the most appropriate covariance structure for each variable. Inferences for each variable were based on the model with the smallest Bayesian information criterion value. Satterthwaite's procedure was used to approximate the denominator degrees of freedom. When *F*-tests were significant ($P < 0.05$), the PDIFF option in SAS was used to compare means. These statistical methods are consistent with those described previously (St-Pierre and Sylvester, 2005).

Univariate procedures (Proc Univariate; SAS) were used to determine whether the data deviated significantly from a normal distribution. Except for monocytes, eosinophils, and basophils/100 WBC, there was no evidence that data for the other variables deviated significantly from a normal distribution. However, monocyte, eosinophil, and basophil data were not transformed for analysis because plotted deviations from normality were small; the average difference between treatments in greatest and least number of monocytes was only 2.4 cells/100 WBC; and the differences for eosinophils and basophils were less than 1 cell/100 WBC.

Least squares means are presented, instead of simple, unadjusted means. The Proc Mixed method of SAS predicts standard errors (S.E.) for each least squares mean. Because of the large number of means from this experiment, within a variable the standards errors for the means of the

Table 3
Least squares means for fatty acid concentrations (μg/mL) in plasma from milk replacer fed lambs that received 1 g twice daily of either soybean oil, safflower oil, or fish oil

Fatty acid	Soybean oil	Safflower oil	Fish oil	Day 7	Day 28	Pooled S.E.[a]
$C_{12:0}$	0.67	0.68	0.64	0.67	0.65	0.02
$C_{14:0}$	2.71	2.74	3.18	3.00	2.75	0.12
$C_{14:1}$	0.03	0.03	0.04	0.03	0.04	0.003
$C_{15:0}$	0.67	0.64	0.74	0.68	0.69	0.05
$C_{16:0}$	98.42	96.41	8.11	103.34	98.62	2.95
trans $C_{16:1}$[b]	0.11	0.10	0.13	0.10	0.13	0.01
$C_{16:1}$	5.89	5.88	7.06	6.32	6.23	0.22
$C_{17:0}$	2.04	1.95	2.26	2.19	1.98	0.21
$C_{18:0}$[b]	135.93	133.11	146.18	151.13	125.68	3.68
trans-6 $C_{18:1}$ or *trans*-7 $C_{18:1}$[c]	1.32	1.43	1.48	1.46	1.36	0.05
trans-9 $C_{18:1}$[b]	3.11	2.59	3.02	2.66	3.16	0.11
trans-10 $C_{18:1}$	0.62	0.74	0.68	0.68	0.69	0.03
trans-11 $C_{18:1}$[b]	1.31	1.27	1.30	1.11	1.48	0.07
trans-16 $C_{18:1}$[b]	0.16	0.17	0.20	0.19	0.16	0.01
trans-12 $C_{18:1}$ or *cis*-7 $C_{18:1}$[c]	0.50	0.56	0.54	0.54	0.54	0.02
trans-13 *cis*-6 $C_{18:1}$[b]	7.14	6.54	6.46	6.20	7.22	0.24
cis-9 $C_{18:1}$	175.69	171.40	199.77	184.45	180.12	6.26
cis-11 $C_{18:1}$	17.78	17.38	20.11	18.71	18.13	0.59
cis-12 $C_{18:1}$	1.20	1.22	1.28	1.18	1.28	0.06
cis-13 $C_{18:1}$	1.08	1.10	1.30	1.20	1.12	0.04
cis-15 $C_{18:1}$[b]	1.51	1.46	1.63	1.70	1.37	0.04
$C_{18:2(n-6)}$	394.28	393.27	423.58	424.24	383.18	10.27
cis-9, *trans*-12 $C_{18:2}$[b]	7.98	7.34	7.28	6.98	8.09	0.26
cis-9, *trans*-11 CLA[d]	0.09	0.12	0.13	0.13	0.09	0.01
trans-8, *cis*-10 CLA[d]	0.06	0.05	0.04	0.06	0.04	0.006
trans-10, *cis*-12 CLA[d]	0.006	0.002	0.0001	0.003	0.003	0.001
trans-9, *trans*-11 CLA[b,d]	0.54	0.49	0.52	0.47	0.57	0.01
trans-11, *trans*-13 CLA[b,d]	0.08	0.07	0.10	0.10	0.08	0.005
Other *trans*, *trans* CLA[d,e]	0.27[g]	0.26[g]	0.43[h]	0.30	0.34	0.01
$C_{18:3(n-3)}$	8.18	7.72	8.85	8.48	8.02	0.23
$C_{20:0}$[b]	2.13	2.04	2.30	2.37	1.94	0.08
$C_{20:3(n-3)}$	4.64	4.74	5.52	5.26	4.68	0.15
$C_{20:4(n-6)}$	68.07	67.34	73.08	69.60	69.40	2.33
$C_{20:5(n-3)}$[f]	6.13	3.51	13.63	6.79	8.72	1.03
$C_{22:1}$	0.97	1.26	1.20	1.28	1.01	0.09
$C_{22:4(n-6)}$[f]	6.06	6.30	5.49	6.17	5.73	0.21
$C_{22:5(n-3)}$	11.23	11.28	13.58	12.02	12.03	0.46
$C_{22:6(n-3)}$[f]	10.63	10.65	21.48	12.02	16.49	0.86
$C_{24:0}$	0.04	0.06	0.07	0.05	0.06	0.008
$C_{24:1}$	0.22	0.14	0.53	0.24	0.35	0.12
Total	978.95	964.53	1084.15	1043.76	974.66	26.39
$\sum(n-3)$[f]	40.51	37.87	62.95	44.61	49.61	2.19
$\sum(n-6)$	468.40	467.01	502.31	499.98	458.50	12.22
$\sum(n-3):\sum(n-6)$[f]	0.09	0.08	0.13	0.09	0.11	0.003
$\sum$CLA[e]	1.06[g]	1.00[g]	1.23[h]	1.06	1.13	0.03

Means without at least one superscript letter (g, h) in common are different ($P < 0.05$).

[a] Root mean-square error and degrees of freedom – 1 were used to calculate pooled standard errors (S.E.).

[b] The effect of day of blood sample collection was significant ($P < 0.05$).

[c] Fatty acids coeluted, and the peak could have been either one of the two.

[d] CLA = conjugated linoleic acid. All of these are $C_{18:2}$ lipids.

[e] The effect of oil treatment was significant ($P < 0.05$).

[f] The day × oil treatment interaction was significant ($P < 0.05$). For each significant day × oil treatment interaction, the day 28 value for the fish oil treatment differed ($P < 0.01$ to $P < 0.001$) from all other treatment–day combinations.

individual values were similar, and attaching a standard error to every mean would make the tables difficult to interpret, pooled standard errors are shown.

3. Results

3.1. Fatty acids

Fish oil treatment increased plasma concentration of total $n-3$ fatty acids ($P < 0.001$) and the total $n-3$:total $n-6$ fatty acid ratio ($P < 0.001$). However, the oil treatment × day interaction was significant ($P < 0.02$ and 0.001, respectively) for both variables (Table 3). The interaction seemed to have emerged because day 28 values for fish oil ($n-3$, 73.95 μg/mL; total $n-3$:total $n-6$, 0.15) were greater than they were for all other day–treatment combinations ($n-3$, 41.74 μg/mL; total $n-3$:total $n-6$, 0.09), and total $n-3$ fatty acid concentrations and total $n-3$:total $n-6$ fatty acid ratio did not differ between any combinations of day and soybean oil or safflower oil treatment. Fish

Table 4
Least squares means for unstimulated, concanavalin A (Con A) stimulated, and lipopolysaccharides (LPS) stimulated lymphocyte proliferation (expressed as optical density units, odu), and differential white blood cell counts (expressed as cells per 100 white blood cells) for milk replacer fed lambs that received 1 g twice daily of either soybean oil, safflower oil, or fish oil

Variable	Oil			Day				Pooled S.E.[a]
	Soybean	Safflower	Fish	7	14	21	28	
Unstimulated[b]	1.17	1.28	1.19	0.88[e]	1.19[f]	1.47[f]	1.31[f]	0.05
Con A[b]	1.31	1.52	1.57	1.04[e]	1.51[f]	1.90[g]	1.41[f,h]	0.05
LPS[b]	1.27	1.31	1.48	0.93[e]	1.31[f]	1.75[g]	1.44[f,h]	0.04
Neutropils[c]	43.0[e,f]	37.9[f,g]	38.7[g]	44.1[e]	37.8[f]	37.9[f]	39.5[e,f]	0.97
Lymphocytes[b,d]	46.8[e]	54.2[f,g]	50.9[e,g]	45.8[e]	53.6[f]	52.6[f]	50.5[e,f]	0.97
Monocytes	7.9	5.5	7.4	7.7	6.0	7.2	6.8	0.24
Eosinophils	0.05	0.06	0.09	0.04	0.001	0.08	0.14	0.02
Basophils	2.2	2.2	2.9	2.1	2.1	2.4	3.0	0.17

Means without at least one superscript letter (e, f, g, h) in common are different ($P < 0.05$).
[a] Root mean-square error and degrees of freedom – 1 were used to calculate pooled standard errors (S.E.).
[b] Effect of day of blood sample collection was significant ($P < 0.05$).
[c] Day × oil treatment interaction was significant ($P < 0.05$).
[d] Effect of oil treatment was significant ($P < 0.05$).

oil also increased ($P < 0.05$) plasma concentration of total conjugated linoleic acids (Table 3). Neither oil treatment nor day affected the concentrations of total $n-6$ fatty acids, and the oil treatment × day interaction was not significant (Table 3).

Day affected ($P < 0.05$) the concentration of 11 of the fatty acids that were measured, and treatment affected ($P < 0.05$) the concentration of other *trans*, *trans* CLA (Table 3). The interaction of oil treatment × day was significant ($P < 0.01$) for $C_{20:5(n-3)}$, $C_{22:4(n-6)}$, and $C_{22:6(n-3)}$ (Table 3). The interaction seemed to have resulted because the day 28 values for fish oil ($C_{20:5(n-3)}$, 19.12 μg/mL; $C_{22:4(n-6)}$, 3.76 μg/mL; $C_{22:6(n-3)}$, 28.34 μg/mL) were different ($P < 0.01$ to <0.001) from those for all other day–treatment combinations ($C_{20:5(n-3)}$, 5.48 μg/mL; $C_{22:4(n-6)}$, 6.39 μg/mL; $C_{22:6(n-3)}$, 11.44 μg/mL).

3.2. Body weights and gains

Pen body weight (i.e., weight of ram lamb + weight of ewe lamb per pen) increased ($P < 0.001$) with day of experiment (day 7, 11.9 kg; day 14, 15.1 kg; day 21, 18.2 kg; day 28, 21.2 kg; pooled S.E. = 0.31 kg), but oil treatment and the oil treatment × day interaction were not significant. Oil treatment, day, and the oil treatment × day interaction were not significant for pen body weight gains (3.5 kg; pooled S.E. = 0.10 kg), pen average daily gains (0.5 kg; pooled S.E. = 0.01 kg), pen milk intakes (19.0 kg; pooled S.E. = 0.35 kg), or pen gain:feed ratio (0.18; pooled S.E. = 0.005) measured during three 7-day intervals (i.e., days 7–14; days 14–21; and days 21–28).

The gender × day interaction was significant ($P < 0.01$) for weekly body weights of the lambs, but neither oil treatment nor interactions with oil treatment were significant. The interaction seemed to be because, for each day, rams were heavier ($P < 0.01$) than ewe lambs. On days 7, 14, 21, and 28 of the experiment, ram lambs weighted 6.3, 8.2, 10.0, and 11.9 kg, respectively. On days 7, 14, 21, and 28 of the experiment, ewe lambs weighted 5.7, 7.4, 8.9, and 10.2 kg, respectively; pooled S.E. = 0.04 kg.

3.3. Lymphocyte proliferation and white blood cell counts

Day affected unstimulated ($P < 0.001$), Con A stimulated ($P < 0.001$), and LPS stimulated ($P < 0.001$) lymphocyte proliferation; lymphocyte proliferation for cells collected on days 14, 21, and 28 was greater than it was for cells collected on day 7 (Table 4). Oil treatment and the oil treatment × day interaction were not significant (Table 4) for lymphocyte proliferation.

For neutrophils per 100 WBC, the treatment × day interaction was significant ($P < 0.05$). The interaction seemed to be because neutrophil numbers per 100 WBC were greater ($P < 0.05$) on day 7 than they were on days 14, 21, and 28. Plus, neutrophil numbers per 100 WBC were greater ($P < 0.05$) for soybean oil than for fish oil treatment, while neutrophil numbers for safflower oil treatment did not differ from numbers for soybean or fish oil treatment.

The effects of treatment and day on numbers of lymphocytes per 100 WBC were significant ($P < 0.01$ and <0.05, respectively; Table 4). Lymphocyte numbers per 100 WBC were greater for safflower oil than for soybean oil treatment, but lymphocyte numbers for fish oil treatment were intermediate and did not differ from numbers for safflower oil or soybean oil treatment (Table 4). Lymphocyte numbers per 100 WBC were less on day 7 than on days 14 and 21, and day 28 numbers were intermediate (Table 4). For monocytes, eosinophils, and basophils, oil treatment, day, and the oil treatment × day interaction were not significant (Table 4).

4. Discussion

Twice daily oral administration of fish oil to orphaned, milk replacer fed lambs increased plasma concentrations of total, long chain $n-3$ PUFA and conjugated linoleic acids, and it increased the total $n-3$:total $n-6$ ratio. This indicates that the actual effect of fish oil treatment was consistent with the desired effect and that assumption of the experimental model was satisfied. Data from other studies indicate that feeding fish oil will increase long chain $n-3$ PUFA and the total $n-3$:total $n-6$ ratio in sheep plasma, muscle, and milk (Kitessa et al., 2003; Ponnampalam et

al., 2001; Capper et al., 2007). In addition, feeding fish oil has increased conjugated linoleic acids in sheep and cattle (Whitlock et al., 2002; Capper et al., 2007). Functional ruminants were used for the studies referenced in the previous two sentences, but the lambs used in the current study had never been given access to solid feed and were effectively nonruminants during the experiment. However, fish oil supplementation of swine diets has increased long chain $n-3$ PUFA and the total $n-3$:total $n-6$ ratio (Rooke et al., 2001a,b). Safflower oil had no detectable effect on fatty acid concentrations, and fatty acid values for safflower oil and soybean oil supplemented lambs were not different.

Despite the clear effects of fish oil on fatty acid concentrations, fish oil treatment did not affect any measure of growth of the lambs in the current study. In studies in which growing pigs were fed fish oil, the fish oil treatment did not affect growth rates, unless pigs were given an immune challenge with lipopolysaccharides (Carroll et al., 2003; Gaines et al., 2003; Liu et al., 2003). Fish oil supplementation of pigs seemed to attenuate the negative effects of lipopolysaccharides on growth rates (Gaines et al., 2003; Liu et al., 2003). In studies in which growing lambs or pigs suckled dams that received fish oil in their diets, so that the milk was enriched with long chain $n-3$ PUFA, fish oil did not improve lamb or pig growth rates (Rooke et al., 2001a,b; Capper et al., 2007). Thus, even though one should not expect fish oil supplementation to improve the growth performance of healthy, milk replacer fed lambs, the data from this study indicate that fish oil supplementation should not reduce growth performance.

Fish oil supplementation of lambs in the current study did not enhance, or inhibit, any measure of lymphocyte proliferation in vitro. This is consistent with some studies, but not with others. Indeed, the effects of fish oil treatment on immune responses may justifiably be characterized as variable, and the responses seem to vary with species, age, and stressor being evaluated (Kelley and Daudu, 1993; Grimble, 2001; Sijben and Calder, 2007). Fish oil supplementation of apparently healthy beef steers seemed to enhance in vitro lymphocyte proliferation, and fish oil supplementation of human infants seemed to enhance the rate of immune maturation, without affecting immune activation (Wistuba et al., 2005; Damsgaard et al., 2007). Studies with mice indicated that fish oil supplementation did not affect the in vitro response of lymphocytes to Con A or phytohemagglutinin, but fish oil seemed to enhance in vivo proliferation of aged lymphocytes (VanMeter et al., 1994). Other studies indicate that fish oil suppresses immune functions (Fritsche et al., 1991; Mills et al., 2005). Nevertheless, fish oil seems to consistently improve immune functions in immunosuppressed animals (Grimble, 2001; Mills et al., 2005; Gabler and Spurlock, 2008). The health status of the lambs in the current study was not quantified systematically. However, except for the seven lambs that were removed from the study, the health of the lambs, based on observations made at least twice daily throughout the study, did not seem compromised. Perhaps fish oil supplementation would benefit milk replacer reared lambs that have become immunosuppressed (for review of potential mechanisms, see Grimble, 2001; Mills et al., 2005; Gabler and Spurlock, 2008).

All measures of lymphocyte proliferation in vitro were increased after lambs were 7 days of age. This is consistent with data on postnatal maturation of pulmonary antimicrobial defenses in lambs (Weiss et al., 1986). Even though the oil treatment × day interaction was significant for neutrophils and oil treatment and day were significant for lymphocyte numbers per 100 WBC, the physiological significance of these effects is difficult to determine. Lymphocyte numbers changed with day of age, but not with any identifiable event, and this seems similar to other data for neonatal lambs (Peña et al., 2004). Neutrophil numbers were greater on day 7 than on other days, but again this change did not seem to be associated with an identifiable event. Fish oil did not seem to affect lymphocyte numbers per 100 WBC, but neutrophil numbers per 100 WBC were slightly less in fish oil, compared with soybean oil, treated lambs. Based on our previous research (Ramadan et al., 1997; Lewis, 2003; Wulster-Radcliffe et al., 2003), differential white blood cell counts are often difficult to interpret, unless some specific event, such as bacterial challenge, can be identified.

5. Conclusion

Even though twice daily oral administration of fish oil to orphaned, milk replacer fed lambs increased plasma concentrations of total, long chain $n-3$ PUFA and increased the total $n-3$:total $n-6$ ratio, these changes produced no measurable benefits to the lambs in the current study. Based on clinical signs, all of the lambs were healthy when they were assigned to the study. With the exception of 7 of the 120 lambs, the lambs remained healthy throughout the study. A number of reports indicate that fish oil supplementation does not seem especially beneficial to young animals unless they become immunocompromised or immunochallenged. There were no visual signs or immune cell responses to indicate that the lambs in this study were immunocompromised or immunochallenged. Perhaps, based on the scientific literature, the lambs would have benefited from their enhanced $n-3$ PUFA status if they had been immunochallenged. Indeed, a study to determine whether fish oil supplementation would benefit immunocompromised or immunochallenged milk replacer reared lambs seems warranted. Until such data are available, the costs associated with fish oil supplementation of milk replacer reared lambs do not seem justified.

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